The Therapeutic Potential of PTEN Modulation

McLoughlin, N.M.; Mueller, C.; Grossmann, T.N.

published in
Cell Chemical Biology
2018

DOI (link to publisher)
10.1016/j.chembiol.2017.10.009

document version
Publisher's PDF, also known as Version of record

Cell Chemical Biology
Perspective

The Therapeutic Potential of PTEN Modulation: Targeting Strategies from Gene to Protein

Niall M. McLoughlin,[1] Carolin Mueller,[1] and Tom N. Grossmann[1,2,*]
[1]Department of Chemistry and Pharmaceutical Sciences, VU University Amsterdam, 1081 HZ Amsterdam, the Netherlands
[2]Chemical Genomics Centre of the Max Planck Society, 44227 Dortmund, Germany
*Correspondence: t.n.grossmann@vu.nl
https://doi.org/10.1016/j.chembiol.2017.10.009

Two decades have passed since the discovery of the tumor suppressor, PTEN. A multitude of biological functions have since been revealed, suggesting potential therapeutic applications for both PTEN activation (e.g., cancer) and inhibition (e.g., neuroregeneration). Nevertheless, PTEN's therapeutic suitability has been called into question due to its "risky" profile as a tumor suppressor. To evaluate PTEN function and its various roles in disease a number of molecules have so far been developed. However, intrinsic problems associated with phosphatase inhibition and PTEN's complex regulation via post-translational modifications hinder straightforward access to selective modulators. For this reason, central questions associated with PTEN targeting remain unanswered. In this perspective, we summarize current PTEN-targeting strategies and discuss potential approaches to modulate its functional dose, considering all stages of PTEN biogenesis from direct protein modulation to the targeting of relevant miRNAs as well as the *PTEN* gene and mRNA.

Introduction

In 1997, a phosphatase-encoding gene, which upon deletion induced aggressive tumorigenesis across kidney, breast and prostate cell lines (Li et al., 1997; Steck et al., 1997), was identified. Phosphatase and Tensin Homolog on Chromosome 10 (PTEN) was quickly validated as an essential tumor suppressor and over the course of the next two decades, characterized as a ubiquitous modulator of cell growth and proliferation, features mainly associated with its lipid phosphatase activity (Salmena, 2016). After *p53*, *PTEN* is the second most commonly mutated tumor suppressor in human cancer with some 30% of tumors harboring somatic, PTEN mutations (Nakamura et al., 2000; Simpson and Parsons, 2001). In addition, PTEN dysregulation has been implicated not only in cancer but in a wide variety of other non-oncogenic illnesses, including Alzheimer's disease and autism (Knafo et al., 2016; Zhou and Parada, 2012).

PTEN at the 10q23 locus is under the control of various transcription factors such as EGR-1, p53, ATF2, PPARγ (Correia et al., 2014). Other proteins such as HES-1, C-Jun, and NF-κB transcriptionally repress PTEN, while an additional layer of epigenetic repression has also been reported to occur via methylation of the *PTEN* promoter or via histone deacetylase-containing complexes such as PML/RARα or NuRD/Mi2 (Correia et al., 2014; Lu et al., 2009; Noguera et al., 2016). Upon transcription, *PTEN* mRNA undergoes splicing and is translocated to the cytosol where it becomes a target for miRNA-guided degradation (Sharrard and Maitland, 2000) (Figure 1). Prominent PTEN-targeting miRNAs include proto-oncogenic miR-19, miR-21, and miR-221 (Garofalo et al., 2009; Meng et al., 2007; Olive et al., 2009). As an added layer of regulatory complexity, several long non-coding RNAs (lncRNAs) purportedly sequester miRNAs from binding to *PTEN* mRNA, however the biological significance of this mechanism remains controversial (Poliseno et al., 2010; Tay et al., 2011; Thomson and Dinger, 2016).

PTEN is a protein containing 403 amino acids with a number of additional proteoforms (Malaney et al., 2017). It fulfills its main tumor suppressor activity at the plasma membrane by dephosphorylating phosphatidylinositol (3,4,5)-trisphosphate (PIP_3, Figure 1) to phosphatidylinositol-4,5-bisphosphate (PIP_2) (Song et al., 2012) thereby counteracting phosphoinositide 3-kinase (PI3K) signaling (Jean and Kiger, 2014). Among others (Rowland et al., 2011), PTEN inhibits AKT-dependent pathways such as GSK3 (metabolic stimulation), FOXO (cell cycle progression), BAD/MDM2 (cell survival), and the mTOR axis (protein synthesis) (Song et al., 2012). Critically, PTEN's phosphatase activity and subcellular localization are dynamically controlled by post-translational modifications (PTMs) (Bermúdez Brito et al., 2015), examples include phosphorylation, ubiquitination, oxidation, acetylation, and sumoylation (Bermúdez Brito et al., 2015). Phosphorylation of PTEN's C-tail promotes electrostatic interactions with its membrane-binding region, resulting in a conformationally closed state with reduced membrane association and phosphatase activity (Silva et al., 2008) (Figure 1). While not fully elucidated, autodephosphorylation contributes to the conformational re-opening of PTEN (Rahdar et al., 2009; Tibarewal et al., 2012). Monoubiquitination by NEDD4-1 promotes nuclear import, whereas polyubiquitination by the same enzyme or others marks PTEN for proteasomal degradation (Leslie et al., 2016). Certain PTMs can also mediate direct inhibition of phosphatase activity as seen when PTEN's catalytic cysteine is oxidized by reactive oxygen species (Cho et al., 2004; Lee et al., 2015).

Aside from PIP_3 dephosphorylation and autodephosphorylation, PTEN recognizes other protein substrates and is involved in a number of protein-protein interactions (PPIs) (Myers et al., 1997; Worby and Dixon, 2014). PPIs involving

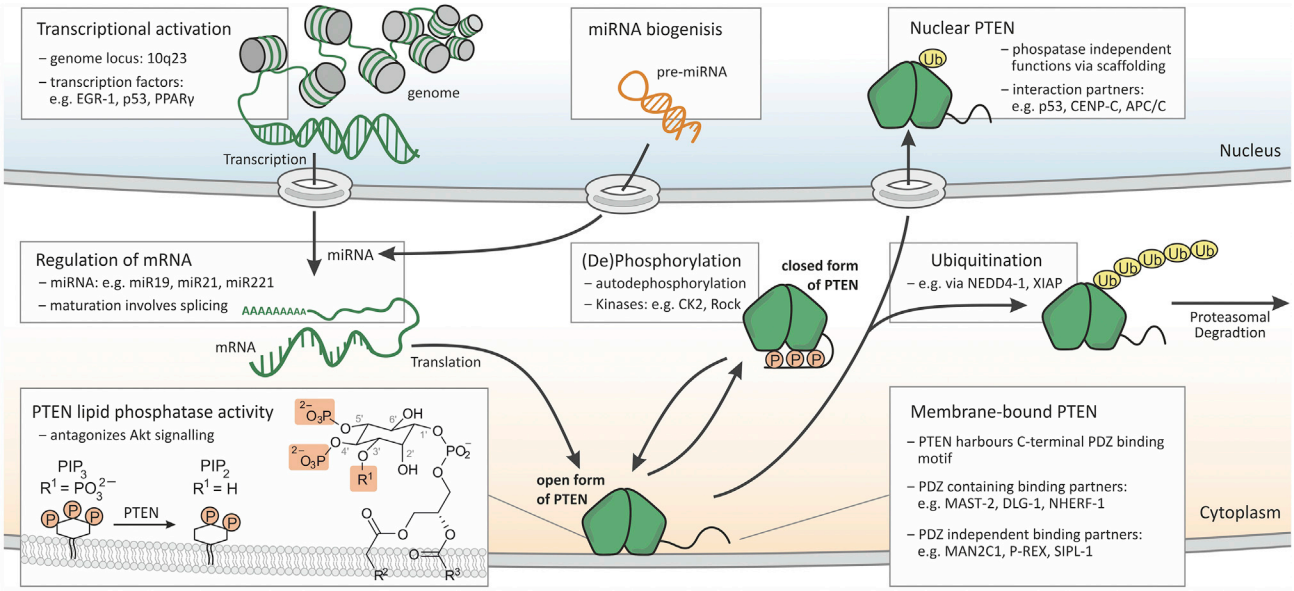

Figure 1. Schematic Overview of PTEN Biogenesis and Regulation

Located at chromosome 10q23, *PTEN* is transcribed under the influence of various transcription factors, including EGR-1, p53 and PPARγ. Upon transcription, *PTEN* mRNA is spliced and becomes a target for a number of miRNAs, including miR-21. Translation yields the PTEN protein, a membrane-active lipid phosphatase that primarily dephosphorylates PIP_3 to provide PIP_2 thereby antagonizing AKT signaling. Membrane-localized PTEN also engages in a number of protein-protein interactions (PPIs) with PDZ-containing proteins through its C-tail domain. PTMs modulate both PTEN's enzymatic activity and cellular localization. Via phosphorylation of its C-tail, PTEN enters a conformationally closed state, which promotes membrane dissociation. In contrast, mono-ubiquitination can promote nuclear localization where PTEN may act via phosphatase-dependent or -independent mechanisms. Poly-ubiquitination promotes PTEN degradation.

PTEN's PDZ-binding motif at the plasma membrane often enhance PTEN's phosphatase activity (e.g., interactions with DLG1, MAST2, or NHERF1) while PPIs involving PTEN's C2 or phosphatase domains are known to repress enzymatic activity (e.g., PREX2a, SIPL1, or Man2C1 interactions) (Worby and Dixon, 2014). In the nucleus, PTEN-mediated PPIs enhance the activity of numerous protein substrates such as the kinetochore-binding protein CENP-C, the E3 ubiquitin ligase APC/C as well as p53 (Worby and Dixon, 2014). In general, these nuclear interactions have been shown to benefit chromosomal stability and healthy cell-cycle progression (Worby and Dixon, 2014). However, it remains unclear whether PTEN's phosphatase activity acts in concert with these effects.

The modulation of PTEN activity and/or protein levels is considered a potential therapeutic strategy for a number of disease states. To describe the overall amount of PTEN capable of engaging in both phosphatase-dependent and -independent activities, the term "functional dose" has been introduced (Berger et al., 2011). Given PTEN's central role in the regulation of cell growth and proliferation, changes to its functional dose trigger profound biological effects that are expected to be of therapeutic use (Figure 2) (Alimonti et al., 2010). A reduction of PTEN's functional dose can promote cell growth and proliferation and is considered beneficial in regenerative medicine, e.g., after stroke or ischemia, and in Alzheimer's disease. However, it is not clear to what extent such strategies may induce cancer formation. On the other hand, increasing PTEN's functional dose and thereby promoting its tumor suppressor activity has been pursued for the development of anti-cancer therapies, the treatment of certain forms of autism as well as cancer predisposition syndromes.

In this perspective, we summarize available strategies for both reduction and increase of functional dose, considering all stages of PTEN biogenesis from direct protein modulation to the targeting of relevant miRNAs as well as the *PTEN* gene and mRNA (Figure 2, bottom). In addition, emerging technologies are highlighted that could provide valuable, PTEN-targeting strategies. We conclude with a general discussion of PTEN's potential as a therapeutic target and the risks associated with its central tumor suppressor function.

Reduction of PTEN's Functional Dose

The reduction of PTEN's functional dose has garnered interest in the context of tissue regeneration, given PTEN's central role as an antagonist of AKT-mediated cell growth and proliferation. In the nervous system where tissue (re)growth is intrinsically protracted, conditional PTEN deletion (and subsequent mTOR activation) has been shown to promote axon regeneration after crush injuries in both optical and spinal neurons (Liu et al., 2010; Park et al., 2008; Sun et al., 2011). Analogously, conditional PTEN deletion benefits cardiomyocyte survival by preventing ischemia and limiting reperfusion (Bird et al., 2014; Ruan et al., 2009). Collectively, these findings have shaped PTEN's profile as a therapeutic target after stroke and nerve injuries. PTEN dose reduction may also find application in the context of Alzheimer's disease. As recently shown, PTEN silencing or inhibition of PTEN membrane localization at synapses affected by β-amyloid aggregation leads to strong reductions in Alzheimer-mediated cognitive deficiencies (Knafo et al., 2016). Approaches to reduce PTEN's functional dose involve direct protein inhibition, the targeting of *PTEN* mRNA, and anti-gene strategies (Figure 2).

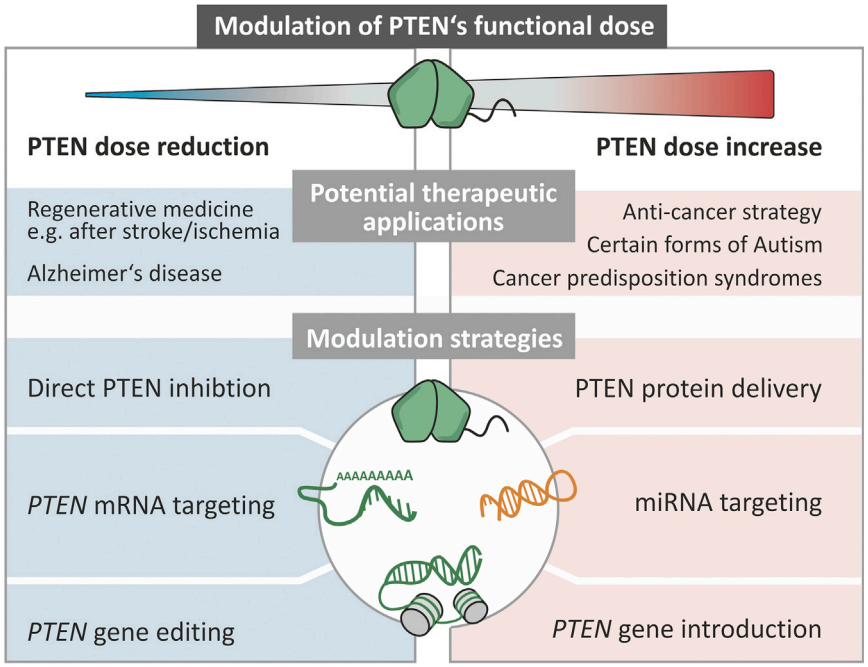

Figure 2. Overview of Potential Therapeutic Applications and Strategies Toward PTEN Modulation

Direct Targeting of PTEN Function

PTEN is translated as a 403 residue protein comprised of a central phosphatase and C2 domain flanked by flexible regulatory tails (Figure 3A) (Lee et al., 1999). The phosphatase domain harbors a highly conserved active site around the catalytic cysteine (C124) within the canonical phosphate-binding loop (P loop: [I/V]HCXXGXXR[S/T]) (Figure 3B) (Lee et al., 1999). Together with its neighboring C2 domain, the phosphatase domain bares a number of surface exposed lysine and arginine residues which facilitate PTEN's association to the plasma membrane (Figure 3A) (Song et al., 2012). Other binding sites include a PIP_2 recognition region within PTEN's N-tail and a PDZ-binding motif at the C terminus (Campbell et al., 2003; Lee et al., 1999). So far, two strategies have been pursued to directly reduce PTEN's functional dose: (1) inhibition of PTEN's phosphatase activity and (2) inhibition of PTEN PPIs.

Inhibition of Phosphatase Activity. Similar to other protein tyrosine phosphatases (PTPs), PTEN has proven a challenging target with only a small number of inhibitors so far developed (Fahs et al., 2016; Stanford and Bottini, 2017). Early PTEN inhibitors originated from a screen of bisperoxidovanadium (bpV) complexes with promiscuous PTP inhibitory activities (Salmena, 2016). Of these, complexes such as bpV-OHpic (Figure 3C) exhibit selectivity for PTEN above PTP-β and PTP-1B, resulting in activation of AKT-dependent signaling in cell-based assays (Schmid et al., 2004). Subsequently, bpV complexes were utilized in a number of *in vivo* studies to inhibit PTEN and induce mTOR-mediated axon regeneration (Christie et al., 2010; Walker and Xu, 2014; Wu et al., 2013). Mechanistically, these complexes act via oxidation of PTEN's catalytic cysteine C124 resulting in the formation of a disulfide bond with closely aligned cysteine C71 (Figure 3D) (Lee et al., 2015). This oxidation is reversible and results in the formation of orthovanadate, which is known to inhibit various other human enzymes (Costa Pessoa et al., 2015). Vanadyl (VO) complexes such as VO-OHpic (Figure 3C), have also been shown to inhibit PTEN phosphatase activity *in vitro* (half maximal inhibitory concentration, 35 nM) while triggering increased PIP_3 levels and AKT translocation in NIH 3T3 fibroblasts (Rosivatz et al., 2006). Unlike bpVs, VO complexes are suggested to inhibit phosphatases through a reversible, non-oxidative mechanism, a feature recently exploited for activity-based, phosphatase imaging probes (Collins et al., 2016). Aside from vanadium-based complexes, SF1670 and the napthoquinone-derived natural product Shikonin have been reported to inhibit PTEN's phosphatase activity (Figure 3E), likely through irreversible mechanisms of action (Garlich et al., 2005; Nigorikawa et al., 2006; Spinelli et al., 2015).

Inhibition of Protein-Protein Interactions. Inhibiting PPIs has also been explored to reduce PTEN functional dose. For this purpose, antagonistic molecules have been derived from PTEN itself. Esteban and co-workers synthesized a membrane-permeable variant of PTEN's PDZ-binding motif which they found to block post-synaptic PTEN membrane localization and reduce AKT/GSK3β-directed signaling in Alzheimer's mouse models (Knafo et al., 2016). Similarly, PDZ-derived peptide antagonists were capable of blocking MAST2-PTEN interactions (Terrien et al., 2012). In addition, other peptides derived from PTEN's phosphatase domain and C-tail have been found to positively influence axon growth and recovery in mice affected by spinal injury (Ohtake et al., 2014). Despite these results, the PTEN specificity of these molecules is intrinsically limited due to the ubiquity of PDZ motifs and recognition elements (Lee and Zheng, 2010). Alternatively, antagonists derived from PTEN interaction partners have been reported such as 3L4F, a truncated peptide from an intracellular loop of the 5-HT_{2C} receptor (5-HT_{2C}R), which was found to prevent PTEN-5-HT_{2C}R complex formation and to decrease PTEN-mediated dephosphorylation both *in vitro* and *in vivo* (Anastasio et al., 2013; Ji et al., 2006).

Targeting PTEN mRNA and PTEN Gene Editing

Inhibition of PTEN biogenesis has been mainly pursued using antisense oligonucleotides (ASOs). Comprised of DNA or non-natural analogs, ASOs target complementary mRNA strands through Watson-Crick base-pairing to prevent translational processing and/or induce nuclease-mediated degradation (Li and Rana, 2014). Initially reported ASOs targeting *PTEN* mRNA were comprised of phosphorothioate backbones and 2′-*O*-methoxy-ethyl (MOE)-modified ribonucleotides, which displayed strong biological profiles, reducing PTEN protein expression by up to 90% in mouse liver tissues (Butler et al., 2002). Further

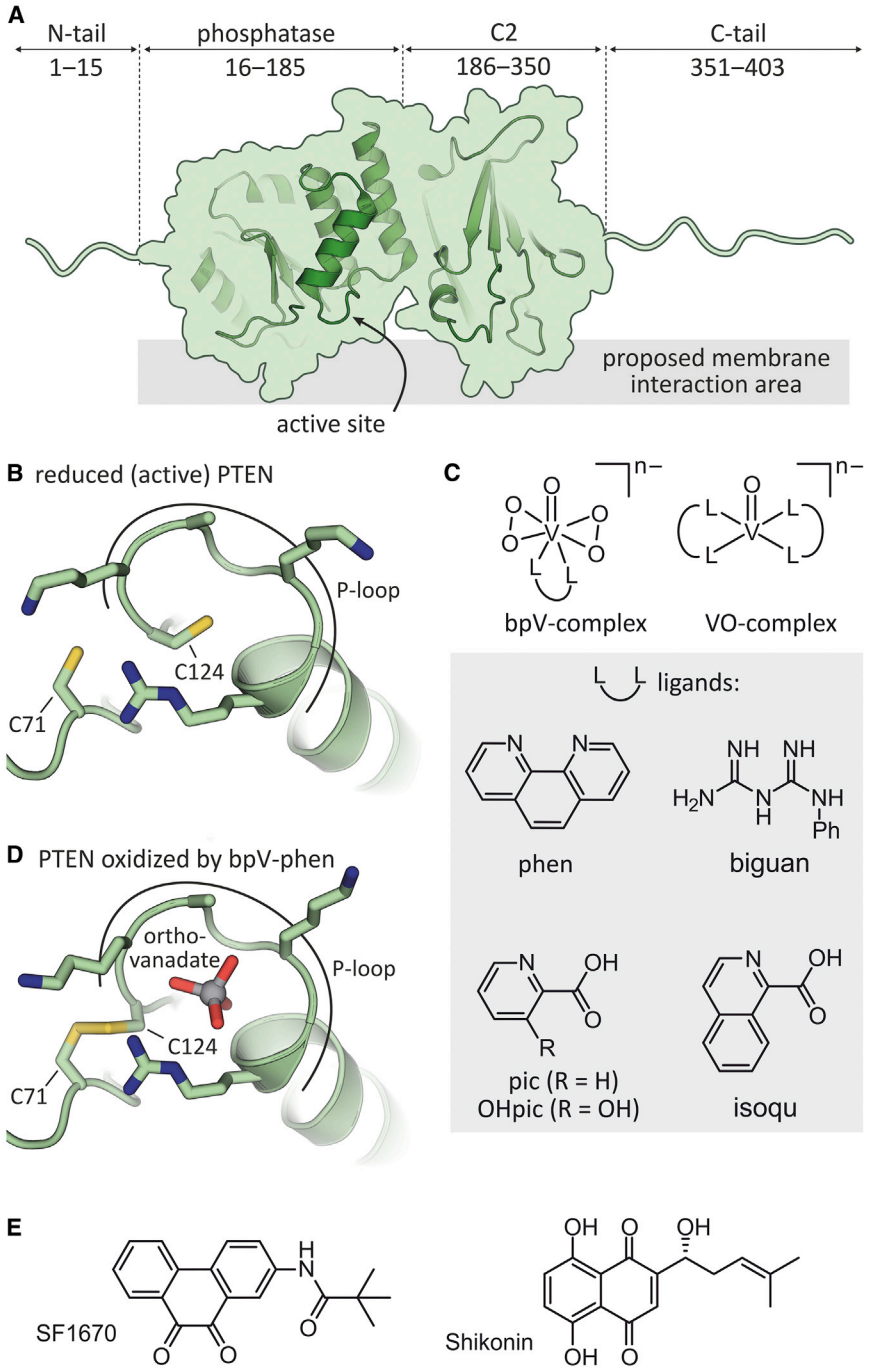

Figure 3. Inhibition of PTEN Phosphatase Activity

(A) Schematic overview of PTEN's structure (derived from crystal structure PDB: 5BZZ) (Lee et al., 2015).

(B) Close up of PTEN's active site not showing a tartrate molecule originating from crystallization buffer (PDB: 5BZZ) (Lee et al., 2015).

(C) Selection of bpV and VO complexes exhibiting PTEN phosphatase inhibition.

(D) Close up of PTEN's active site after bpV-mediated disulfide bridge formation (PDB: 5BUG) harboring orthovanadate (Lee et al., 2015).

(E) Chemical structures of PTEN inhibitors.

elaboration using locked nucleic acid (LNA) analogs yielded even greater potency but also led to hepatotoxicity *in vivo* (Swayze et al., 2007). Aside from ASOs, the therapeutic application of RNAi strategies (e.g., short interfering RNA [siRNA] and short hairpin RNA [shRNA]) has been explored with the support of specialized delivery modalities (Kanasty et al., 2013; Krishnamurthy et al., 2008). In one example, the use of lipopeptide nanoparticles allowed for the targeted delivery of *PTEN*-specific siRNAs to liver hepatocytes in mice, where PTEN expression levels were reduced by up to 80% (Dong et al., 2014). In the case of *PTEN*-targeting shRNA, delivery via tyrosine-mutated adeno-associated virus (AAV) vectors induced mTOR-mediated axon regeneration in rat optic nerves (Huang et al., 2017).

Finally, rather than targeting *PTEN*'s gene products, proof-of-principle studies to directly inhibit *PTEN* transcription (antigene strategies) have been reported (Cox et al., 2015; Gutilla and Steward, 2016). By flanking *PTEN* with *LoxP* sequences, He and co-workers deleted *PTEN* in mouse sensimotor cortices using an AAV-Cre-mediated system to induce mTOR-mediated corticospinal regeneration after injury (Liu et al., 2010). Notably, even after long-term (>12 months) *PTEN* deletion, cortical neurons displayed healthy phenotypes and did not display tumorigenesis (Gutilla et al., 2016).

Reduction of PTEN Dose: Conclusions and Outlook

The design of selective and potent PTEN inhibitors remains an unresolved issue in drug discovery. Similar to other PTPs, this is mainly due to the highly polar nature of PTEN's active site, which hampers the development of cell-permeable inhibitors (Stanford and Bottini, 2017). For that reason, it is not surprising that reported PTEN inhibitors often follow a covalent and rather unselective mode of action involving the targeting of cysteine 124. Building on the susceptibility of this cysteine, covalent fragment-based screening could be envisioned to identify more selective, irreversible inhibitors (Kathman et al., 2014; Ruddraraju and Zhang, 2017). Recent examples of allosteric and bivalent PTP inhibitors that target alternative sites may also open new options for the development of selective PTEN inhibitors (Zhang, 2017). Separately, the disruption of PTEN PPIs has shown potential in providing a means to selectively inhibit PTEN in defined disease contexts, potentially allowing for intervention with specific functions rather than the entire PIP_3-dependent signaling network. While preliminary examples have mainly focused on

antagonistic unmodified peptides, the use of peptidomimetic inhibitors should provide analogs with higher bioactivity (Pelay-Gimeno et al., 2015). In this respect, macrocyclic structures, derived from irregular loop-like binding epitopes are particularly interesting (Glas et al., 2014; Wiedmann et al., 2017). Addressing the low cellular uptake of peptide-derived structures, lessons learned from cell-penetrating peptides (Brock, 2014; Dietrich et al., 2017) or strategies that use smaller ligands that only form the product upon target engagement should be considered (Brauckhoff et al., 2014; Jaegle et al., 2017). To fully evaluate the potential of such strategies, a more detailed investigation of PTEN's interactome is required. In this context, the use of mixed modalities or the stabilization of certain PTEN PPIs could provide novel probes or potentially more selective agents (Thiel et al., 2012; Valeur et al., 2017). On a translational level, ASOs and RNAi strategies that target *PTEN* mRNA have proven useful for the modulation of PTEN's functional dose. However, well-documented delivery issues associated with these techniques are the main limiting factor for broader applications (Dowdy, 2017). Finally, the rise of novel gene editing techniques such as CRISPR/Cas 9, C2c2, or Cpf1 could offer potentially very efficient access to PTEN depletion in a tissue-specific manner provided that current delivery and selectivity issues have been addressed (Nakade et al., 2017).

Increase of PTEN's Functional Dose

PTEN is a quasi-insufficient tumor suppressor referring to the fact that partial PTEN inactivation can already promote tumorigenesis (Carracedo et al., 2011). PTEN inactivation is seen in a variety of cancers with COSMIC listing over 3,000 *PTEN* mutations in 30 different tissue types. The bulk of these somatic mutations either disable PTEN's active site or alter its C2 domain to prevent membrane binding (Forbes et al., 2015). Therefore, increasing or re-instating PTEN's functional dose has been proposed as an anti-cancer strategy. Increasing its functional dose may also find application in other diseases related to PTEN inactivation, such as certain forms of autism or autism spectrum disorders where germline mutations in *PTEN* lead to increased synapse growth, manifesting in synaptic hyperactivity and excitability (Takeuchi et al., 2013; Knafo and Esteban, 2017). PTEN germline mutations can also lead to cancer predisposition syndromes such as Cowden disease, Bannayan-Riley-Ruvalcaba syndrome and Proteus syndrome (Arch et al., 1997; Hollander et al., 2011; Liaw et al., 1997). For that reason, increasing its functional dose is expected to benefit such patients. An increase of PTEN's functional dose is a particularly challenging endeavor, and depending on the disease context, this may require different strategies. So far, PTEN protein delivery, miRNA targeting, and gene editing have been pursued (Figure 2).

PTEN Protein Delivery

Besides antibodies, peptides, and hormones, a growing list of protein-based therapeutics have received regulatory approval in recent years (Kinch, 2015). While many issues have been overcome, therapeutic protein delivery remains challenging due to degradation and low membrane permeability (Mitragotri et al., 2014). Nonetheless, some studies indicate that the intracellular delivery of certain PTEN variants may be feasible. In one such approach, exogenous introduction of PTEN, functionalized with cationic lipidoid nanoparticles, promoted apoptosis of PTEN-null, prostate cancer cells (PC-3) via the antagonism of AKT signaling (Altinoglu et al., 2016). Fluorescence microscopy of labeled PTEN nanoparticles confirmed intracellular delivery (Altinoglu et al., 2016). Similarly, it was found that silica nanoparticles also promote PTEN's cellular uptake (Arora and Ghosh, 2016). Alternatively, it was reported that a cell-permeable, translational variant of PTEN (PTEN-long) can inhibit PI3K signaling and induce tumor cell apoptosis *in vitro* as well as regression of PTEN-null xenograft tumors in mice (Hopkins et al., 2013).

Inhibition of PTEN*-Targeting miRNAs*

As *PTEN* mRNA is subject to translational repression by a host of miRNAs, miRNA inhibition provides a route to increase PTEN's functional dose. miRNAs are short, non-coding RNAs that are transcribed as part of large stem-loop-containing pri-miRNAs (Figure 4A). Pri-miRNAs are recognized by the RNase Drosha to provide smaller, hairpin-like sequences known as pre-miRNAs (Figure 4A). Upon nuclear export, pre-miRNAs undergo cleavage via the RNase Dicer to generate mature miRNA duplexes of ca. 21 bases in length with short unpaired terminal sequences (Figure 4A). One of the two strands of mature miRNA (guide strand) is then incorporated into the RNA-induced silencing complex (RISC) to recognize complementary or closely related mRNA sequences to block translation and/or promote mRNA degradation (Figure 4A) (Bartel, 2009; Lin and Gregory, 2015). RISC-associated miRNAs often recognize multiple mRNA targets due to their short length. Resultantly, many *PTEN*-targeting miRNAs also regulate other genes: e.g., miR-19 (*BIM*, *BCL2*, *Prkaa2*), miR-21 (*PDC4*, *RECK*, *TPM1*), and miR-221 (*BMF*, *BBC3/PUMA*, *CDKN1B*) (Krichevsky and Gabriely, 2009; Lupini et al., 2013; Mavrakis et al., 2010). In cancer, aberrantly transcribed miRNAs that dysregulate the mRNA of tumor suppressors and oncogenes have been termed oncomiRs (Esquela-Kerscher and Slack, 2006). One such oncomiR is the PTEN-targeting, miR-21, implicated in the progression of hepatocellular cancer, lung cancer, and colorectal cancer (Meng et al., 2007; Xiong et al., 2014; Zhang et al., 2010). miR-21 is a prime target for therapeutic application and its inhibition has been pursued intensively. For that reason, we focus here on miR-21 targeting as a means of increasing PTEN's functional dose.

Direct Inhibition of Mature miR-21. To target mature miR-21 guide strands (red in Figure 4B), a number of antisense oligonucleotides have been developed and are referred to as anti-miR-21s (Li and Rana, 2014). Following initial reports of 2′-OMe anti-miR-21s (Figure 4C), Isis Pharmaceuticals performed a comprehensive screen of anti-miR-21 backbone modifications (Cheng et al., 2005; Davis et al., 2006). While both 2′-*O*-Me and 2′-*O*-MOE-modified derivatives (Figure 4C) showed comparable miR-21 affinity in biochemical assays, 2′-*O*-MOE anti-miR exhibited superior, cell-based miR-21 inhibition likely due to its higher nuclease stability (Davis et al., 2006). In separate studies, short phosphorothioate-based LNAs (Figure 4C) were used to engage the seed region of miR-21 with sub-nanomolar affinity resulting in long-lasting miR-21 inhibition *in vivo* (Obad et al., 2011). As a variation on this concept, anti-miR-21s have recently been assembled using selenomethylene-locked nucleic acids (SeLNA, Figure 4C) to further improve nuclease stability (Nahar et al., 2016a, 2016b). While the above-mentioned ASOs block miR-21, alternative approaches have aimed to degrade miR-21 (Lennox and Behlke, 2011). For this purpose, hybrid anti-miRs

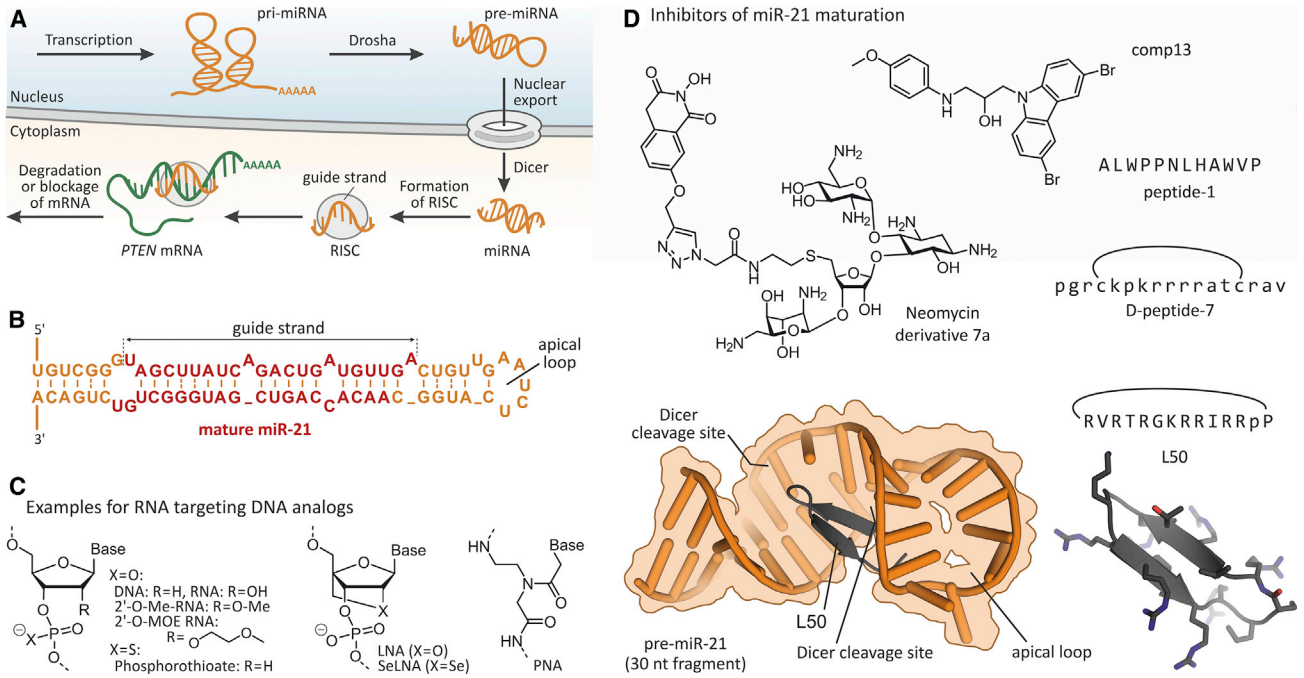

Figure 4. Inhibition of *PTEN*-targeting miRNAs
(A) miRNA biogenesis and function involving pri- and pre-miRNA precursors that are processed by Drosha and Dicer, respectively.
(B) Sequence of precursor stem loop with mature miR-21 (red) and guide strand highlighted.
(C) Examples of oligonucleotide analogs used to construct miR-21-targeting ASOs.
(D) Chemical structures of miR-21 targeting molecules including NMR structure of L50 bound to truncated pre-miR-21 (30 nt, PDB: 5UZZ) (Shortridge et al., 2017).

containing both unmodified DNA and chemically modified building blocks have been employed to allow for RNase H degradation of miR-21 (Davis et al., 2006; Lennox and Behlke, 2010). In addition, catalytically active oligonucleotides have been developed that are capable of cleaving complementary single-stranded RNA. For example, hammerhead ribozymes were engineered to deplete miR-21 levels in human glioblastoma cells, resulting in increase of PTEN protein levels (Belter et al., 2016; Patutina et al., 2017).

Inhibition of miR-21 Maturation. Rather than directly engaging miRNAs, the inhibition of miRNA maturation has also been pursued (Schmidt, 2014; Shortridge and Varani, 2015). miRNA precursors are challenging therapeutic targets, owing to their flexibility, charged character, and lack of atypical ligand recognition motifs (Schmidt, 2014). While in principle, anti-miRs (such as those discussed above) can engage the sequences of miRNA precursors (e.g., pre-miR-21), they usually exhibit substantially lower affinities due to the self-complementarity of precursor hairpin structures. Alternatively, two classes of small molecules have been identified that display general RNA-binding tendencies. These include saccharide-derived structures that often target RNA loops as well as bulges, and aromatic scaffolds that intercalate within RNA duplexes (Thomas and Hergenrother, 2008; Velagapudi and Disney, 2013). For pre-miR-21 (Figure 4B), binding by bioactive aminoglycosides such as streptomycin, neomycin, and kanamycin has been reported, showing relatively low inhibition of Dicer cleavage (Bose et al., 2012; Yan et al., 2017). To increase potency, bifunctional derivatives combining pre-miR-21 binder neomycin with a small-molecule inhibitor of Dicer have been developed. By virtue of conjugation, the neomycin derivative 7a (Figure 4D) saw an increase in Dicer inhibition by more than two orders of magnitude and was capable of selectively reducing miR-21 levels in cell-based assays (Yan et al., 2017). Alternatively, small-molecule screening campaigns for pre-miR-21 binders or maturation inhibitors have proven feasible, yielding a few relatively low affinity tool compounds (Connelly et al., 2017; Gumireddy et al., 2008; Naro et al., 2015; Shi et al., 2013). In one recent example, a microarray screen of 20,000 compounds provided comp13 (Figure 4D), which displays low micromolar affinity for pre-miR-21 and was found to inhibit Dicer cleavage in biochemical assays (Connelly et al., 2017).

To inhibit challenging biological targets such as pre-miRNA, peptides have recently attracted attention due to their large binding surfaces and unique conformational propensities (Pai et al., 2012). Using phage display, a linear, 12-mer peptide (peptide-1, Figure 4D) with high affinity for pre-miR-21 (K_d = 13 nM) was found to inhibit Dicer processing in cell-based assays while also upregulating PTEN expression (Bose et al., 2015). In a separate study, mirror-image phage display of disulfide bridged peptides against pre-miR-21 was performed to address the inherent protease susceptibility of peptides. In this setup, L-peptides are screened against "mirror" RNA (L-enantiomer of RNA) with corresponding hit sequences then used as templates for D-peptides to bind natural D-RNA. In the case of pre-miR-21, this approach generated D-peptide-7 (Figure 4D), which displayed moderate affinity for pre-miR-21 in biochemical assays (Sakamoto et al., 2017). Recently, pre-miR-21 was also screened against a small

cyclic peptide library based on the RNA-binding domain of the bovine immunodeficiency virus Tat sequence. Using electromobility shift assays, cyclic peptide L50 (Figure 4D) was identified and later characterized as a selective, high-affinity pre-miR-21 binder (K_d = 0.2 μM). In addition to inhibiting Dicer cleavage *in vitro,* a nuclear magnetic resonance (NMR) structure of pre-mir-21 in complex with peptide L50 was elucidated (Figure 4D). Interestingly, L50 binds to the major groove of the stem region adjacent to the apical loop thereby blocking sites of Dicer cleavage (Shortridge et al., 2017).

PTEN Gene Introduction

One final strategy that has briefly been described toward increasing PTEN's functional dose is *PTEN* gene introduction. First reported by Tanaka et al. in 2000, *PTEN* was exogenously introduced via an adenoviral vector into PTEN-null human bladder cancer cells where it was found to suppress tumor cell growth through the abrogation of AKT signaling (Tanaka et al., 2000). Subsequent studies involving human colorectal and prostate cancer cells yielded similar results (Saito et al., 2003; Tanaka et al., 2005). Moreover, for human bladder and colorectal cancer, gene editing successfully reduced tumor growth in xenograft mice (Saito et al., 2003; Tomioka et al., 2008). Notably, the introduction of additional PTEN genes into cells with physiological PTEN levels had only little effect, supporting the therapeutic applicability of this approach (Tanaka and Grossman, 2003). Even more remarkably, mice with systemically elevated PTEN ("Super-PTEN" mutants) display healthy phenotypes and are characterized with heightened resistance to cancer (Garcia-Cao et al., 2012).

Increase of PTEN Dose: Conclusions and Outlook

Overall, increasing PTEN functional dose represents an alternative to inhibitors of PI3K (responsible for PIP3 production at the membrane), AKT, or mTOR, most of which have faced efficacy issues related to therapeutic resistance (Porta et al., 2014). One way to achieve PTEN dose increase could involve the development of PTEN agonists or molecules that activate pathogenic PTEN variants. As shown for the discovery of allosteric SHIP-1 phosphatase activators, the screening of natural product libraries against PTEN could provide a novel source of such compounds (Ong et al., 2007). Another plausible PTEN activation strategy involves the design of bifunctional compounds to localize PTEN at the plasma membrane (Stiller et al., 2017) thereby promoting its phosphatase activity. As the case for protein delivery has been made above, engineered variants of PTEN with enhanced phosphatase activity, such as the recently reported ePTEN (Nguyen et al., 2014), may support a therapeutic application.

In regard to direct miR-21 targeting, the full breadth of available DNA analogs (e.g., PNA and moropholinos) has yet to be explored and can yield novel ASO-based inhibitors (van Rooij and Kauppinen, 2014). To circumvent the delivery issues associated with ASOs, however, the targeting of miRNA maturation is becoming increasingly investigated. With respect to small molecules, the utilization of dynamic combinatorial libraries or covalent inhibitor strategies can be expected to facilitate the discovery of novel pre-miR-21 ligands in the future (Connelly et al., 2016). Moreover, given recent achievements in phage display (Pai et al., 2012) and protein design (Chen et al., 2016) it is clear that peptide-derived structures also hold the potential to provide selective pri- or pre-miR-21 binders. From initial screens, peptidomimetics such as peptoid ligands have exhibited suitability for this purpose (Chirayil et al., 2009; Diaz et al., 2014). The screening of alternative modalities such as aptamers appears to show similar promise and may be an alternative source of novel ligands (Kabza and Sczepanski, 2017). Having focused on miR-21, additional PTEN-targeting oncomiRs should also be considered and used to probe the suitability of miR-21 in the context of its other targets. Finally, with respect to gene therapy, many hurdles need to be overcome with regard to undesired immunogenicity and non-specific genetic modification (Kotterman et al., 2015). Once more, recent advances in CRISPR/Cas 9 gene editing and other systems provide a potentially powerful platform to correct for somatic *PTEN* mutations. However, considerable efforts are still required before such techniques see widespread therapeutic applications (Dai et al., 2016; Nakade et al., 2017; Xue et al., 2014).

Concluding Remarks

It is now 20 years since PTEN's discovery. Within the first decade, many fundamental insights into PTEN's phosphatase activity, its structure, and tumor suppressor function were pieced together. Passing into the second decade, large strides were made in understanding the extent and subtleties of PTEN regulation from the transcriptional level to PTMs. Having all these insights available, still many more questions regarding PTEN biology remain to be answered, e.g., What are the precise, nuclear roles of PTEN? How do phosphatase independent functions contribute to its tumor suppressor activity? What is the biological significance of PTEN's growing list of isoforms and translational variants? To what degree does epigenetic regulation affect PTEN functional dose? How is PTEN dose affected by oncomiR networks?

The third decade can be expected to elucidate these remaining aspects of PTEN biology but also to contribute therapeutically relevant targeting strategies. In this respect, it is essential to identify disease contexts in which PTEN targeting is systemically tolerated. Particularly in regard to reducing its functional dose, PTEN's "risky" profile as a tumor suppressor has to be considered. While recent studies suggest a possible therapeutic window for *PTEN* dose reduction without triggering tumorigenesis, dysfunctional outcomes of *PTEN* deletion in the past have raised questions over its therapeutic suitability (Gutilla et al., 2016; Liu et al., 2010; Pun et al., 2012; Williams et al., 2015). To address these issues, potent and selective inhibitors are required that allow selective, short-term PTEN inhibition preferably in a tissue-specific manner. In our view, such compounds will likely arise from protein- or mRNA-targeting approaches. In particular, when considering the availability of new targeting paradigms such as selective ribosomal inhibitors, spliceosome inhibitors, or targeted protein degradation approaches (e.g., PROTACS) (Cromm and Crews, 2017; León et al., 2017; Lintner et al., 2017). Undoubtedly, increasing PTEN's functional dose is of prime interest as an anti-cancer strategy but is also extremely challenging, mainly due to a lack of PTEN-activating agents and general shortcomings related to miRNA targeting. It can be expected that all levels of PTEN biogenesis and regulation must be taken into consideration when aiming for therapeutically relevant PTEN dose reduction. In addition, it remains to be seen if

certain oncogenic PTEN variants are tractable as drug targets themselves, and if PTEN's post-translationally modified derivatives can be selectively modulated.

Taken together, we can conclude that PTEN is certainly an intriguing therapeutic target but that more efforts are required to define relevant indications both for an increase and a reduction of PTEN's functional dose. To address this question, chemical tools are required that allow selective modulation of the various PTEN functions. This process should be curiosity driven and consider all options, not only the protein's phosphatase activity, PPIs, and PTMs but also the *PTEN* gene and mRNA, including regulating miRNAs. For that reason, it is desirable that the different subfields move closer together and that resulting tool compounds are more rigorously tested in biologically relevant systems. With that in mind, one can certainly expect the chemical biology community to provide selective agents that may finally open up new pathways toward PTEN's pharmacological intervention.

ACKNOWLEDGMENTS

We are grateful for support from the European Research Council (ERC; ERC starting grant no. 678623), AstraZeneca, Bayer CropScience, Bayer HealthCare, Boehringer Ingelheim, Merck KGaA and the Max Planck Society.

REFERENCES

Alimonti, A., Carracedo, A., Clohessy, J.G., Trotman, L.C., Nardella, C., Egia, A., Salmena, L., Sampieri, K., Haveman, W.J., Brogi, E., et al. (2010). Subtle variations in Pten dose determine cancer susceptibility. Nat. Genet. *42*, 454–458.

Altinoglu, S.A., Wang, M., Li, K.Q., Li, Y., and Xu, Q. (2016). Intracellular delivery of the PTEN protein using cationic lipidoids for cancer therapy. Biomater. Sci. *4*, 1773–1780.

Anastasio, N.C., Gilbertson, S.R., Bubar, M.J., Agarkov, A., Stutz, S.J., Jeng, Y., Bremer, N.M., Smith, T.D., Fox, R.G., Swinford, S.E., et al. (2013). Peptide inhibitors disrupt the serotonin 5-HT2C receptor interaction with phosphatase and tensin homolog to allosterically modulate cellular signaling and behavior. J. Neurosci. *33*, 1615–1630.

Arch, E.M., Goodman, B.K., Van Wesep, R.A., Liaw, D., Clarke, K., Parsons, R., McKusick, V.A., and Geraghty, M.T. (1997). Deletion of PTEN in a patient with Bannayan-Riley-Ruvalcara syndrome suggests allelism with Cowden disease. Am. J. Med. Genet. *71*, 489–493.

Arora, N., and Ghosh, S.S. (2016). Functional characterizations of interactive recombinant PTEN-silica nanoparticles for potential biomedical applications. RSC Adv. *6*, 114944–114954.

Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. Cell *136*, 215–233.

Belter, A., Rolle, K., Piwecka, M., Fedoruk-Wyszomirska, A., Naskręt-Barciszewska, M.Z., and Barciszewski, J. (2016). Inhibition of miR-21 in glioma cells using catalytic nucleic acids. Sci. Rep. *6*, 24516.

Berger, A.H., Knudson, A.G., and Pandolfi, P.P. (2011). A continuum model for tumour suppression. Nature *476*, 163–169.

Bermúdez Brito, M., Goulielmaki, E., and Papakonstanti, E.A. (2015). Focus on PTEN regulation. Front. Oncol. *5*, 166.

Bird, G.H., Irimia, A., Ofek, G., Kwong, P.D., Wilson, I.A., and Walensky, L.D. (2014). Stapled HIV-1 peptides recapitulate antigenic structures and engage broadly neutralizing antibodies. Nat. Struct. Mol. Biol. *21*, 1058–1067.

Bose, D., Jayaraj, G., Suryawanshi, H., Agarwala, P., Pore, S.K., Banerjee, R., and Maiti, S. (2012). The tuberculosis drug streptomycin as a potential cancer therapeutic: inhibition of miR-21 function by directly targeting its precursor. Angew. Chem. Int. Ed. *51*, 1019–1023.

Bose, D., Nahar, S., Rai, M.K., Ray, A., Chakraborty, K., and Maiti, S. (2015). Selective inhibition of miR-21 by phage display screened peptide. Nucleic Acids Res. *43*, 4342–4352.

Brauckhoff, N., Hahne, G., Yeh, J.T.-H., and Grossmann, T.N. (2014). Protein-templated peptide ligation. Angew. Chem. Int. Ed. *53*, 4337–4340.

Brock, R. (2014). The uptake of arginine-rich cell-penetrating peptides: putting the puzzle together. Bioconjug. Chem. *25*, 863–868.

Butler, M., McKay, R.A., Popoff, I.J., Gaarde, W.A., Witchell, D., Murray, S.F., Dean, N.M., Bhanot, S., and Monia, B.P. (2002). Specific inhibition of PTEN expression reverses hyperglycemia in diabetic mice. Diabetes *51*, 1028–1034.

Campbell, R.B., Liu, F., and Ross, A.H. (2003). Allosteric activation of PTEN phosphatase by phosphatidylinositol 4,5-bisphosphate. J. Biol. Chem. *278*, 33617–33620.

Carracedo, A., Alimonti, A., and Pandolfi, P.P. (2011). PTEN level in tumor suppression: how much is too little? Cancer Res. *71*, 629–633.

Chen, Y., Yang, F., Zubovic, L., Pavelitz, T., Yang, W., Godin, K., Walker, M., Zheng, S., Macchi, P., and Varani, G. (2016). Targeted inhibition of oncogenic miR-21 maturation with designed RNA-binding proteins. Nat. Chem. Biol. *12*, 717–723.

Cheng, A.M., Byrom, M.W., Shelton, J., and Ford, L.P. (2005). Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res. *33*, 1290–1297.

Chirayil, S., Chirayil, R., and Luebke, K.J. (2009). Discovering ligands for a microRNA precursor with peptoid microarrays. Nucleic Acids Res. *37*, 5486–5497.

Cho, S.-H., Lee, C.-H., Ahn, Y., Kim, H., Kim, H., Ahn, C.-Y., Yang, K.-S., and Lee, S.-R. (2004). Redox regulation of PTEN and protein tyrosine phosphatases in H_2O_2-mediated cell signaling. FEBS Lett. *560*, 7–13.

Christie, K.J., Webber, C.A., Martinez, J.A., Singh, B., and Zochodne, D.W. (2010). PTEN inhibition to facilitate intrinsic regenerative outgrowth of adult peripheral axons. J. Neurosci. *30*, 9306–9315.

Collins, J., Cilibrizzi, A., Fedorova, M., Whyte, G., Mak, L.H., Guterman, I., Leatherbarrow, R., Woscholski, R., and Vilar, R. (2016). Vanadyl complexes with dansyl-labelled di-picolinic acid ligands: synthesis, phosphatase inhibition activity and cellular uptake studies. Dalton Trans. *45*, 7104–7113.

Connelly, C.M., Moon, M.H., and Schneekloth, J.S. (2016). The emerging role of RNA as a therapeutic target for small molecules. Cell Chem. Biol. *23*, 1077–1090.

Connelly, C.M., Boer, R.E., Moon, M.H., Gareiss, P., and Schneekloth, J.S. (2017). Discovery of inhibitors of MicroRNA-21 processing using small molecule microarrays. ACS Chem. Biol. *12*, 435–443.

Correia, N.C., Gírio, A., Antunes, I., Martins, L.R., and Barata, J.T. (2014). The multiple layers of non-genetic regulation of PTEN tumour suppressor activity. Eur. J. Cancer *50*, 216–225.

Costa Pessoa, J., Garribba, E., Santos, M.F.A., and Santos-Silva, T. (2015). Vanadium and proteins: uptake, transport, structure, activity and function. Coord. Chem. Rev. *301–302*, 49–86.

Cox, D.B.T., Platt, R.J., and Zhang, F. (2015). Therapeutic genome editing: prospects and challenges. Nat. Med. *21*, 121–131.

Cromm, P.M., and Crews, C.M. (2017). Targeted protein degradation: from chemical biology to drug discovery. Cell Chem. Biol. *24*, 1181–1190.

Dai, W.-J., Zhu, L.-Y., Yan, Z.-Y., Xu, Y., Wang, Q.-L., and Lu, X.-J. (2016). CRISPR-Cas9 for in vivo gene therapy: promise and hurdles. Mol. Ther. Nucleic Acids *5*, e349.

Davis, S., Lollo, B., Freier, S., and Esau, C. (2006). Improved targeting of miRNA with antisense oligonucleotides. Nucleic Acids Res. *34*, 2294–2304.

Diaz, J.P., Chirayil, R., Chirayil, S., Tom, M., Head, K.J., and Luebke, K.J. (2014). Association of a peptoid ligand with the apical loop of pri-miR-21 inhibits cleavage by Drosha. RNA *20*, 528–539.

Dietrich, L., Rathmer, B., Ewan, K., Bange, T., Heinrichs, S., Dale, T.C., Schade, D., and Grossmann, T.N. (2017). Cell permeable stapled peptide

inhibitor of Wnt signaling that targets β-catenin protein-protein interactions. Cell Chem. Biol. *24*, 958–968.

Dong, Y., Love, K.T., Dorkin, J.R., Sirirungruang, S., Zhang, Y., Chen, D., Bogorad, R.L., Yin, H., Chen, Y., Vegas, A.J., et al. (2014). Lipopeptide nanoparticles for potent and selective siRNA delivery in rodents and nonhuman primates. Proc. Natl. Acad. Sci. USA *111*, 3955–3960.

Dowdy, S.F. (2017). Overcoming cellular barriers for RNA therapeutics. Nat. Biotechnol. *35*, 222–229.

Esquela-Kerscher, A., and Slack, F.J. (2006). Oncomirs — microRNAs with a role in cancer. Nat. Rev. Cancer *6*, 259–269.

Fahs, S., Lujan, P., and Köhn, M. (2016). Approaches to study phosphatases. ACS Chem. Biol. *11*, 2944–2961.

Forbes, S.A., Beare, D., Gunasekaran, P., Leung, K., Bindal, N., Boutselakis, H., Ding, M., Bamford, S., Cole, C., Ward, S., et al. (2015). COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res. *43*, D805–D811.

Garcia-Cao, I., Song, M.S., Hobbs, R.M., Laurent, G., Giorgi, C., de Boer, V.C.J., Anastasiou, D., Ito, K., Sasaki, A.T., Rameh, L., et al. (2012). Systemic elevation of PTEN induces a tumor-suppressive metabolic state. Cell *149*, 49–62.

Garlich, J.R., Durden, D.L., Georgiadis, T.M., Su, J., Peng, X., and Smith, T.C. (2005). PTEN Inhibitors. Patent WO2005097119, filed April 6, 2005, and published October 20, 2005.

Garofalo, M., Di Leva, G., Romano, G., Nuovo, G., Suh, S.S., Ngankeu, A., Taccioli, C., Pichiorri, F., Alder, H., Secchiero, P., et al. (2009). miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell *16*, 498–509.

Glas, A., Bier, D., Hahne, G., Rademacher, C., Ottmann, C., and Grossmann, T.N. (2014). Constrained peptides with target-adapted cross-links as inhibitors of a pathogenic protein-protein interaction. Angew. Chem. Int. Ed. *53*, 2489–2493.

Gumireddy, K., Young, D.D., Xiong, X., Hogenesch, J.B., Huang, Q., and Deiters, A. (2008). Small-molecule inhibitors of MicroRNA miR-21 function. Angew. Chem. Int. Ed. *47*, 7482–7484.

Gutilla, E.A., and Steward, O. (2016). Selective neuronal PTEN deletion: can we take the brakes off of growth without losing control? Neural Regen. Res. *11*, 1201–1203.

Gutilla, E.A., Buyukozturk, M.M., and Steward, O. (2016). Long-term consequences of conditional genetic deletion of PTEN in the sensorimotor cortex of neonatal mice. Exp. Neurol. *279*, 27–39.

Hollander, M.C., Blumenthal, G.M., and Dennis, P. (2011). PTEN loss in the continuum of common cancers, rare syndromes and mouse models. Nat. Rev. Cancer *11*, 289–301.

Hopkins, B.D., Fine, B., Steinbach, N., Dendy, M., Rapp, Z., Shaw, J., Pappas, K., Yu, J.S., Hodakoski, C., Mense, S., et al. (2013). A secreted PTEN phosphatase that enters cells to alter signaling and survival. Science *341*, 399–402.

Huang, Z., Hu, Z., Xie, P., and Liu, Q. (2017). Tyrosine-mutated AAV2-mediated shRNA silencing of PTEN promotes axon regeneration of adult optic nerve. PLoS One *12*, e0174096.

Jaegle, M., Wong, E.L., Tauber, C., Nawrotzky, E., Arkona, C., and Rademann, J. (2017). Protein-templated fragment ligations-from molecular recognition to drug discovery. Angew. Chem. Int. Ed. *56*, 7358–7378.

Jean, S., and Kiger, A.A. (2014). Classes of phosphoinositide 3-kinases at a glance. J. Cell Sci. *127*, 923–928.

Ji, S.-P., Zhang, Y., Van Cleemput, J., Jiang, W., Liao, M., Li, L., Wan, Q., Backstrom, J.R., and Zhang, X. (2006). Disruption of PTEN coupling with 5-HT2C receptors suppresses behavioral responses induced by drugs of abuse. Nat. Med. *12*, 324–329.

Kabza, A.M., and Sczepanski, J.T. (2017). An L-RNA Aptamer with expanded chemical functionality that inhibits MicroRNA biogenesis. ChemBioChem *18*, 1824–1827.

Kanasty, R., Dorkin, J.R., Vegas, A., and Anderson, D. (2013). Delivery materials for siRNA therapeutics. Nat. Mater. *12*, 967–977.

Kathman, S.G., Xu, Z., and Statsyuk, A.V. (2014). A fragment-based method to discover irreversible covalent inhibitors of cysteine proteases. J. Med. Chem. *57*, 4969–4974.

Kinch, M.S. (2015). An overview of FDA-approved biologics medicines. Drug Discov. Today *20*, 393–398.

Knafo, S., and Esteban, J.A. (2017). PTEN: local and global modulation of neuronal function in health and disease. Trends Neurosci. *40*, 83–91.

Knafo, S., Sánchez-Puelles, C., Palomer, E., Delgado, I., Draffin, J.E., Mingo, J., Wahle, T., Kaleka, K., Mou, L., Pereda-Perez, I., et al. (2016). PTEN recruitment controls synaptic and cognitive function in Alzheimer's models. Nat. Neurosci. *19*, 443–453.

Kotterman, M.A., Chalberg, T.W., and Schaffer, D.V. (2015). Viral vectors for gene therapy: translational and clinical outlook. Annu. Rev. Biomed. Eng. *17*, 63–89.

Krichevsky, A.M., and Gabriely, G. (2009). miR-21: a small multi-faceted RNA. J. Cell. Mol. Med. *13*, 39–53.

Krishnamurthy, M., Schirle, N.T., and Beal, P.A. (2008). Screening helix-threading peptides for RNA binding using a thiazole orange displacement assay. Bioorg. Med. Chem. *16*, 8914–8921.

Lee, H.-J., and Zheng, J.J. (2010). PDZ domains and their binding partners: structure, specificity, and modification. Cell Commun. Signal. *8*, 8.

Lee, C.U., Hahne, G., Hanske, J., Bange, T., Bier, D., Rademacher, C., Hennig, S., and Grossmann, T.N. (2015). Redox modulation of PTEN phosphatase activity by hydrogen peroxide and bisperoxidovanadium complexes. Angew. Chem. Int. Ed. *54*, 13796–13800.

Lee, J.O., Yang, H., Georgescu, M.M., Di Cristofano, A., Maehama, T., Shi, Y., Dixon, J.E., Pandolfi, P., and Pavletich, N.P. (1999). Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell *99*, 323–334.

Lennox, K.A., and Behlke, M.A. (2010). A direct comparison of anti-microRNA oligonucleotide potency. Pharm. Res. *27*, 1788–1799.

Lennox, K.A., and Behlke, M.A. (2011). Chemical modification and design of anti-miRNA oligonucleotides. Gene Ther. *18*, 1111–1120.

León, B., Kashyap, M.K., Chan, W.C., Krug, K.A., Castro, J.E., La Clair, J.J., and Burkart, M.D. (2017). A challenging pie to splice: drugging the spliceosome. Angew. Chem. Int. Ed. *56*, 12052–12063.

Leslie, N., Kriplani, N., Hermida, M., Alvarez-Garcia, V., and Wise, H. (2016). The PTEN protein: cellular localization and post-translational regulation. Biochem. Soc. Trans. *44*, 273–278.

Li, Z., and Rana, T.M. (2014). Therapeutic targeting of microRNAs: current status and future challenges. Nat. Rev. Drug Discov. *13*, 622–638.

Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., et al. (1997). PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science *275*, 1943–1947.

Liaw, D., Marsh, D.J., Li, J., Dahia, P.L., Wang, S.I., Zheng, Z., Bose, S., Call, K.M., Tsou, H.C., Peacocke, M., et al. (1997). Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat. Genet. *16*, 64–67.

Lin, S., and Gregory, R.I. (2015). MicroRNA biogenesis pathways in cancer. Nat. Rev. Cancer *15*, 321–333.

Lintner, N.G., McClure, K.F., Petersen, D., Londregan, A.T., Piotrowski, D.W., Wei, L., Xiao, J., Bolt, M., Loria, P.M., Maguire, B., et al. (2017). Selective stalling of human translation through small-molecule engagement of the ribosome nascent chain. PLoS Biol. *15*, e2001882.

Liu, K., Lu, Y., Lee, J.K., Samara, R., Willenberg, R., Sears-Kraxberger, I., Tedeschi, A., Park, K.K., Jin, D., Cai, B., et al. (2010). PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat. Neurosci. *13*, 1075–1081.

Lu, J., Jeong, H., Kong, N., Yang, Y., Carroll, J., Luo, H.R., Silberstein, L.E., Ma, Y., and Chai, L. (2009). Stem cell factor SALL4 represses the transcriptions of PTEN and SALL1 through an epigenetic repressor complex. PLoS One *4*, 1–13.

Lupini, L., Bassi, C., Ferracin, M., Bartonicek, N., D'Abundo, L., Zagatti, B., Callegari, E., Musa, G., Moshiri, F., Gramantieri, L., et al. (2013). miR-221 affects multiple cancer pathways by modulating the level of hundreds messenger RNAs. Front. Genet. *4*, 1–9.

Malaney, P., Uversky, V.N., and Davé, V. (2017). PTEN proteoforms in biology and disease. Cell. Mol. Life Sci. *74*, 2783–2794.

Mavrakis, K.J., Wolfe, A.L., Oricchio, E., Palomero, T., de Keersmaecker, K., McJunkin, K., Zuber, J., James, T., Khan, A.A., Leslie, C.S., et al. (2010). Genome-wide RNA-mediated interference screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic leukaemia. Nat. Cell Biol. *12*, 372–379.

Meng, F., Henson, R., Wehbe-Janek, H., Ghoshal, K., Jacob, S.T., and Patel, T. (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology *133*, 647–658.

Mitragotri, S., Burke, P.A., and Langer, R. (2014). Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. Nat. Rev. Drug Discov. *13*, 655–672.

Myers, M.P., Stolarov, J.P., Eng, C., Li, J., Wang, S.I., Wigler, M.H., Parsons, R., and Tonks, N.K. (1997). P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc. Natl. Acad. Sci. USA *94*, 9052–9057.

Nahar, S., Singh, A., Morihiro, K., Moai, Y., Kodama, T., Obika, S., and Maiti, S. (2016a). Systematic evaluation of biophysical and functional characteristics of selenomethylene-locked nucleic acid-mediated inhibition of miR-21. Biochemistry *55*, 7023–7032.

Nahar, S., Kotikam, V., Kumar, V.A., and Maiti, S. (2016b). Inhibition of miR-21 by 3′/5′-serinyl-capped 2′-*O*-methyl RNA interspersed with 2′-*O*-(2-amino-3-methoxypropyl) uridine units. Nucleic Acid Ther. *26*, 327–334.

Nakade, S., Yamamoto, T., and Sakuma, T. (2017). Cas9, Cpf1 and C2c1/2/3? What's next? Bioengineered *8*, 265–273.

Nakamura, N., Ramaswamy, S., Vazquez, F., Signoretti, S., Loda, M., and Sellers, W.R. (2000). Forkhead transcription factors are critical effectors of cell death and cell cycle arrest downstream of PTEN. Mol. Cell. Biol. *20*, 8969–8982.

Naro, Y., Thomas, M., Stephens, M.D., Connelly, C.M., and Deiters, A. (2015). Aryl amide small-molecule inhibitors of microRNA miR-21 function. Bioorg. Med. Chem. Lett. *25*, 4793–4796.

Nguyen, H.-N., Yang, J.-M., Afkari, Y., Park, B.H., Sesaki, H., Devreotes, P.N., and Iijima, M. (2014). Engineering ePTEN, an enhanced PTEN with increased tumor suppressor activities. Proc. Natl. Acad. Sci. USA *111*, E2684–E2693.

Nigorikawa, K., Yoshikawa, K., Sasaki, T., Iida, E., Tsukamoto, M., Murakami, H., Maehama, T., Hazeki, K., and Hazeki, O. (2006). A naphthoquinone derivative, shikonin, has insulin-like actions by inhibiting both phosphatase and tensin homolog deleted on chromosome 10 and tyrosine phosphatases. Mol. Pharmacol. *70*, 1143–1149.

Noguera, N.I., Piredda, M.L., Taulli, R., Catalano, G., Angelini, G., Gaur, G., Nervi, C., Voso, M.T., Lunardi, A., Pandolfi, P.P., et al. (2016). PML/RARa inhibits PTEN expression in hematopoietic cells by competing with PU.1 transcriptional activity. Oncotarget *7*, 66386–66397.

Obad, S., dos Santos, C.O., Petri, A., Heidenblad, M., Broom, O., Ruse, C., Fu, C., Lindow, M., Stenvang, J., Straarup, E.M., et al. (2011). Silencing of microRNA families by seed-targeting tiny LNAs. Nat. Genet. *43*, 371–378.

Ohtake, Y., Park, D., Abdul-Muneer, P.M., Li, H., Xu, B., Sharma, K., Smith, G.M., Selzer, M.E., and Li, S. (2014). The effect of systemic PTEN antagonist peptides on axon growth and functional recovery after spinal cord injury. Biomaterials *35*, 4610–4626.

Olive, V., Bennett, M.J., Walker, J.C., Ma, C., Jiang, I., Cordon-Cardo, C., Li, Q.J., Lowe, S.W., Hannon, G.J., and He, L. (2009). miR-19 is a key oncogenic component of mir-17-92. Genes Dev. *23*, 2839–2849.

Ong, C.J., Ming-Lum, A., Nodwell, M., Ghanipour, A., Yang, L., Williams, D.E., Kim, J., Demirjian, L., Qasimi, P., Ruschmann, J., et al. (2007). Small-molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. Blood *110*, 1942–1949.

Pai, J., Yoon, T., Kim, N.D., Lee, I.S., Yu, J., and Shin, I. (2012). High-throughput profiling of peptide-RNA interactions using peptide microarrays. J. Am. Chem. Soc. *134*, 19287–19296.

Park, K.K., Liu, K., Hu, Y., Smith, P.D., Wang, C., Cai, B., Xu, B., Connolly, L., Kramvis, I., Sahin, M., et al. (2008). Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science *322*, 963–966.

Patutina, O.A., Bichenkova, E.V., Miroshnichenko, S.K., Mironova, N.L., Trivoluzzi, L.T., Burusco, K.K., Bryce, R.A., Vlassov, V.V., and Zenkova, M.A. (2017). miRNases: novel peptide-oligonucleotide bioconjugates that silence miR-21 in lymphosarcoma cells. Biomaterials *122*, 163–178.

Pelay-Gimeno, M., Glas, A., Koch, O., and Grossmann, T.N. (2015). Structure-based design of inhibitors of protein-protein interactions: mimicking peptide binding epitopes. Angew. Chem. Int. Ed. *54*, 8896–8927.

Poliseno, L., Salmena, L., Zhang, J., Carver, B., Haveman, W.J., and Pandolfi, P.P. (2010). A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature *465*, 1033–1038.

Porta, C., Paglino, C., and Mosca, A. (2014). Targeting PI3K/Akt/mTOR signaling in cancer. Front. Oncol. *4*, 1–11.

Pun, R.K., Rolle, I., LaSarge, C., Hosford, B., Rosen, J., Uhl, J., Schmeltzer, S., Faulkner, C., Bronson, S., Murphy, B., et al. (2012). Excessive activation of mTOR in postnatally generated granule cells is sufficient to cause epilepsy. Neuron *75*, 1022–1034.

Rahdar, M., Inoue, T., Meyer, T., Zhang, J., Vazquez, F., and Devreotes, P.N. (2009). A phosphorylation-dependent intramolecular interaction regulates the membrane association and activity of the tumor suppressor PTEN. Proc. Natl. Acad. Sci. USA *106*, 480–485.

van Rooij, E., and Kauppinen, S. (2014). Development of microRNA therapeutics is coming of age. EMBO Mol. Med. *6*, 851–864.

Rosivatz, E., Matthews, J.G., McDonald, N.Q., Mulet, X., Ho, K.K., Lossi, N., Schmid, A.C., Mirabelli, M., Pomeranz, K.M., Erneux, C., et al. (2006). A small molecule inhibitor for phosphatase and tensin homologue deleted on chromosome 10 (PTEN). ACS Chem. Biol. *1*, 780–790.

Rowland, M.M., Bostic, H.E., Gong, D., Speers, A.E., Lucas, N., Cho, W., Cravatt, B.F., and Best, M.D. (2011). Phosphatidylinositol 3,4,5-trisphosphate activity probes for the labeling and proteomic characterization of protein binding partners. Biochemistry *50*, 11143–11161.

Ruan, H., Li, J., Ren, S., Gao, J., Li, G., Kim, R., Wu, H., and Wang, Y. (2009). Inducible and cardiac specific PTEN inactivation protects ischemia/reperfusion injury. J. Mol. Cell. Cardiol. *46*, 193–200.

Ruddraraju, K.V., and Zhang, Z.-Y. (2017). Covalent inhibition of protein tyrosine phosphatases. Mol. BioSyst. *13*, 1257–1279.

Saito, Y., Swanson, X., Mhashilkar, A.M., Oida, Y., Schrock, R., Branch, C.D., Chada, S., Zumstein, L., and Ramesh, R. (2003). Adenovirus-mediated transfer of the PTEN gene inhibits human colorectal cancer growth in vitro and in vivo. Gene Ther. *10*, 1961–1969.

Sakamoto, K., Otake, K., and Umemoto, T. (2017). Discovery of peptidic miR-21 processing inhibitor by mirror image phage display: a novel method to generate RNA binding D-peptides. Bioorg. Med. Chem. Lett. *27*, 826–828.

Salmena, L. (2016). PTEN: history of a tumor suppressor. Methods Mol. Biol. *1388*, 3–11.

Schmid, A.C., Byrne, R.D., Vilar, R., and Woscholski, R. (2004). Bisperoxovanadium compounds are potent PTEN inhibitors. FEBS Lett. *566*, 35–38.

Schmidt, M.F. (2014). Drug target miRNAs: chances and challenges. Trends Biotechnol. *32*, 578–585.

Sharrard, R.M., and Maitland, N.J. (2000). Alternative splicing of the human PTEN/MMAC1/TEP1 gene. Biochim. Biophys. Acta *1494*, 282–285.

Shi, Z., Zhang, J., Qian, X., Han, L., Zhang, K., Chen, L., Liu, J., Ren, Y., Yang, M., Zhang, A., et al. (2013). AC1MMYR2, an inhibitor of dicer-mediated biogenesis of oncomir miR-21, reverses epithelial-mesenchymal transition and suppresses tumor growth and progression. Cancer Res. *73*, 5519–5531.

Shortridge, M.D., and Varani, G. (2015). Structure based approaches for targeting non-coding RNAs with small molecules. Curr. Opin. Struct. Biol. *30*, 79–88.

Shortridge, M.D., Walker, M.J., Pavelitz, T., Chen, Y., Yang, W., and Varani, G. (2017). A macrocyclic peptide ligand binds the oncogenic MicroRNA-21 precursor and suppresses dicer processing. ACS Chem. Biol. *12*, 1611–1620.

Silva, A., Yunes, J.A., Cardoso, B.A., Martins, L.R., Jotta, P.Y., Abecasis, M., Nowill, A.E., Leslie, N.R., Cardoso, A.A., and Barata, J.T. (2008). PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. J. Clin. Invest. *118*, 3762–3774.

Simpson, L., and Parsons, R. (2001). PTEN: life as a tumor suppressor. Exp. Cell Res. *264*, 29–41.

Song, M.S., Salmena, L., and Pandolfi, P.P. (2012). The functions and regulation of the PTEN tumour suppressor. Nat. Rev. Mol. Cell Biol. *13*, 283–296.

Spinelli, L., Lindsay, Y.E., and Leslie, N.R. (2015). PTEN inhibitors: an evaluation of current compounds. Adv. Biol. Regul. *57*, 102–111.

Stanford, S.M., and Bottini, N. (2017). Targeting tyrosine phosphatases: time to end the stigma. Trends Pharmacol. Sci. *38*, 524–540.

Steck, P.A., Pershouse, M.A., Jasser, S.A., Yung, W.K., Lin, H., Ligon, A.H., Langford, L.A., Baumgard, M.L., Hattier, T., Davis, T., et al. (1997). Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet. *15*, 356–362.

Stiller, C., Krüger, D.M., Brauckhoff, N., Schmidt, M., Janning, P., Salamon, H., and Grossmann, T.N. (2017). Translocation of an intracellular protein via peptide-directed ligation. ACS Chem. Biol. *12*, 504–509.

Sun, F., Park, K.K., Belin, S., Wang, D., Lu, T., Chen, G., Zhang, K., Yeung, C., Feng, G., Yankner, B.A., et al. (2011). Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. Nature *480*, 372–375.

Swayze, E.E., Siwkowski, A.M., Wancewicz, E.V., Migawa, M.T., Wyrzykiewicz, T.K., Hung, G., Monia, B.P., and Bennett, C.F. (2007). Antisense oligonucleotides containing locked nucleic acid improve potency but cause significant hepatotoxicity in animals. Nucleic Acids Res. *35*, 687–700.

Takeuchi, K., Gertner, M.J., Zhou, J., Parada, L.F., Bennett, M.V.L., and Zukin, R.S. (2013). Dysregulation of synaptic plasticity precedes appearance of morphological defects in a Pten conditional knockout mouse model of autism. Proc. Natl. Acad. Sci. USA *110*, 4738–4743.

Tanaka, M., and Grossman, H.B. (2003). In vivo gene therapy of human bladder cancer with PTEN suppresses tumor growth, downregulates phosphorylated Akt, and increases sensitivity to doxorubicin. Gene Ther. *10*, 1636–1642.

Tanaka, M., Koul, D., Davies, M.A., Liebert, M., Steck, P.A., and Grossman, H.B. (2000). MMAC1/PTEN inhibits cell growth and induces chemosensitivity to doxorubicin in human bladder cancer cells. Oncogene *19*, 5406–5412.

Tanaka, M., Rosser, C.J., and Grossman, H.B. (2005). PTEN gene therapy induces growth inhibition and increases efficacy of chemotherapy in prostate cancer. Cancer Detect. Prev. *29*, 170–174.

Tay, Y., Kats, L., Salmena, L., Weiss, D., Tan, S.M., Ala, U., Karreth, F., Poliseno, L., Provero, P., Di Cunto, F., et al. (2011). Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell *147*, 344–357.

Terrien, E., Chaffotte, A., Lafage, M., Khan, Z., Prehaud, C., Cordier, F., Simenel, C., Delepierre, M., Buc, H., Lafon, M., et al. (2012). Interference with the PTEN-MAST2 interaction by a viral protein leads to cellular relocalization of PTEN. Sci. Signal. *5*, 1–12.

Thiel, P., Kaiser, M., and Ottmann, C. (2012). Small-molecule stabilization of protein-protein interactions: an underestimated concept in drug discovery? Angew. Chem. Int. Ed. *51*, 2012–2018.

Thomas, J.R., and Hergenrother, P.J. (2008). Targeting RNA with small molecules. Chem. Rev. *108*, 1171–1224.

Thomson, D.W., and Dinger, M.E. (2016). Endogenous microRNA sponges: evidence and controversy. Nat. Rev. Genet. *17*, 272–283.

Tibarewal, P., Zilidis, G., Spinelli, L., Schurch, N., Maccario, H., Gray, A., Perera, N.M., Davidson, L., Barton, G.J., and Leslie, N.R. (2012). PTEN protein phosphatase activity correlates with control of gene expression and invasion, a tumor-suppressing phenotype, but not with AKT activity. Sci. Signal. *5*, ra18.

Tomioka, A., Tanaka, M., De Velasco, M.A., Anai, S., Takada, S., Kushibiki, T., Tabata, Y., Rosser, C.J., Uemura, H., and Hirao, Y. (2008). Delivery of PTEN via a novel gene microcapsule sensitizes prostate cancer cells to irradiation. Mol. Cancer Ther. *7*, 1864–1870.

Valeur, E., Guéret, S.M., Adihou, H., Gopalakrishnan, R., Lemurell, M., Waldmann, H., Grossmann, T.N., and Plowright, A.T. (2017). New modalities for challenging targets in drug discovery. Angew. Chem. Int. Ed. *56*, 10294–10323.

Velagapudi, S.P., and Disney, M.D. (2013). Defining RNA motif-aminoglycoside interactions via two-dimensional combinatorial screening and structure-activity relationships through sequencing. Bioorg. Med. Chem. *21*, 6132–6138.

Walker, C.L., and Xu, X.-M. (2014). PTEN inhibitor bisperoxovanadium protects oligodendrocytes and myelin and prevents neuronal atrophy in adult rats following cervical hemicontusive spinal cord injury. Neurosci. Lett. *573*, 64–68.

Wiedmann, M.M., Tan, Y.S., Wu, Y., Aibara, S., Xu, W., Sore, H.F., Verma, C.S., Itzhaki, L., Stewart, M., Brenton, J.D., et al. (2017). Development of cell-permeable, non-helical constrained peptides to target a key protein-protein interaction in ovarian cancer. Angew. Chem. Int. Ed. *56*, 524–529.

Williams, M.R., DeSpenza, T., Li, M., Gulledge, A.T., and Luikart, B.W. (2015). Hyperactivity of newborn Pten knock-out neurons results from increased excitatory synaptic drive. J. Neurosci. *35*, 943–959.

Worby, C.A., and Dixon, J.E. (2014). PTEN. Annu. Rev. Biochem. *83*, 641–669.

Wu, Z., He, B., He, J., and Mao, X. (2013). Upregulation of miR-153 promotes cell proliferation via downregulation of the PTEN tumor suppressor gene in human prostate cancer. Prostate *73*, 596–604.

Xiong, Y., Zhang, Y.Y., Wu, Y.Y., Wang, X.D., Wan, L.H., Li, L., and Zhou, L.M. (2014). Correlation of over-expressions of miR-21 and Notch-1 in human colorectal cancer with clinical stages. Life Sci. *106*, 19–24.

Xue, W., Chen, S., Yin, H., Tammela, T., Papagiannakopoulos, T., Joshi, N.S., Cai, W., Yang, G., Bronson, R., Crowley, D.G., et al. (2014). CRISPR-mediated direct mutation of cancer genes in the mouse liver. Nature *514*, 380–384.

Yan, H., Bhattarai, U., Guo, Z.-F., and Liang, F.-S. (2017). Regulating miRNA-21 biogenesis by bifunctional small molecules. J. Am. Chem. Soc. *139*, 4987–4990.

Zhang, Z.-Y. (2017). Drugging the undruggable: therapeutic potential of targeting protein tyrosine phosphatases. Acc. Chem. Res. *50*, 122–129.

Zhang, J.G., Wang, J.J., Zhao, F., Liu, Q., Jiang, K., and Yang, G. (2010). MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clin. Chim. Acta *411*, 846–852.

Zhou, J., and Parada, L.F. (2012). PTEN signaling in autism spectrum disorders. Curr. Opin. Neurobiol. *22*, 873–879.